UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

GELLER, 1998a

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 21 December 1998

Subject: Non-parametric Correlations Run on Thyroid Data from Studies Submitted for Evaluation of Perchlorate

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is a report on correlations run on thyroid hormone, TSH, and standard histopathology ratings of thyroid tissue from the Caldwell 14-Day, Subchronic, Rat Developmental Neurotoxicity, and Rabbit Developmental Toxicity studies.

TABLE OF CONTENTS

Report on Correlations of T3, T4, TSH, and Thyroid Histopathology

Introduction: Thyroid hormone homeostasis is largely controlled by a feedback loop between the thyroid gland and the pituitary/hypothalamus. The thyroid gland produces thyroxine (T4) in large quantities and 3,5,3'-triiodothyronine (T3) in lesser amounts. Most T3 is produced by de-iodination of T4 at target tissues. Low levels of circulating T4 and T3 lead to the increased production and release of thyrotropin (TSH) by the pituitary, which in turn elicits increased T4 production. Long periods of elevated TSH can result in hypertrophy and/or hyperplasia of the follicular cells of the thyroid. Because of these relationships, one can expect positive correlations between T3 and T4, negative correlations between T3 or T4 and TSH, positive correlations between TSH and follicular hyperplasia or hypertrophy (FH), and negative correlations between T3 or T4 levels and FH.

The correlations discussed in this report are of two types. Hormone levels are continuous, ratio-scaled values; correlations were computed using the conventional Pearson's r statistic. Correlations between ratio-scaled hormone levels and ordinally-scaled standard histology ratings must be computed using non-parametric correlations. To compare variables from the different scales, it is simplest to recode the data by converting the variable values into rank scores. Spearman's rank order (r_s) correlation computes the correlation between the rankings of two variables. When there are ties in the ranks, as were present in this data set, each value was assigned the mean of the ranks they would otherwise occupy. A correlation coefficient was then computed for the rankings of the variables of interest.

An alternative statistic used for comparing the data sets was Kendall's tau, best thought of as a measure of agreement or concordance between two sets of ranked data. It searches for the number of inversions in two sets of ranked data, i.e., observations are ranked according to the first variable, then re-ranked according to the second, and the number of interchanges that occur is used to compute the statistic.

Statistics were computed using SAS software (PROC RANK, PROC CORR, SAS Institute, Inc., Cary , NC). The Spearman and Kendall statistics produced nearly identical results, and therefore only the Spearman statistic is reported in the following tables. Kendall's tau is included on the data plots.

Caldwell Adult Rat – 14 Days of Dosing (Caldwell, et. al., 1995; Channel, 1998a)

Correlations show a robust relationship between T3/T4, T3/TSH, and T4/TSH. T3 and T4 varied together (Table 1, Figure 1A) while T3 and TSH (Table1) and T4 and TSH (Table 1, Figure 1B) varied inversely.

Hormone levels correlated highly with two standard histopathological ratings of thyroid pathology, FH and decrease in follicular lumen area (LA). The ranks of T3 (Table 2) and T4 levels (Table 2, Figure 1C) vary inversely with severity ratings, i.e., the lower the hormone levels the more severe the histopathology ranking. The ranks of TSH levels varies strongly positively with severity ratings for both FH and decrease in LA (Table 2, Figure 1D). The severity ratings for FH and LA correlate highly with each other (Table 2).

Rat Subchronic Study – Adult Rats (Springborn, 1998)

Table 3 shows the relationships between hormone levels across the 14 and 90 day timepoints, i.e. during dosing. T3 and T4 varied together and were therefore significantly positively correlated. There were significant negative correlations between T3 or T4 and TSH. Table 4 enumerates the significant positive correlations between TSH and FH and significant negative correlations between T3 or T4 and FH (Figure 2A).

After 14 days of dosing , T3 and T4 vary together, but there is an unexpected positive relation between T4 and TSH (Table 5). There is a very strong positive correlation between TSH ranking and FH, and significant negative correlations between T3 or T4 and FH (Table 6) (Figure 2B).

After 90 days of dosing, T3 and T4 were strongly positively correlated and T3 or T4 and TSH were strongly negatively correlated (Table 7). TSH and FH remained positively correlated and T3 or T4 and FH remained negatively correlated (Table 8) (Figure 2C).

Rat Developmental Neurotoxicity – PND5 (Data from Argus, 1998a; York, 1998c)

Data from the Developmental Neurotoxicity study were examined using the litter as the unit of analysis. Where there was more than one pup from a litter, the mean hormone level and severity rating was used. This led. In some cases, to standard histopathology ratings of other than integer values, e.g., 1.5 (Figures 3C - 3F).

T3 and T4 were strongly positively correlated; T4 and TSH were strongly negatively correlated (Table 9, Figures 3A, 3B). T4 was negatively correlated with both histopathology measures (Table 10, Figures 3C, 3D). It was significantly correlated with lumen size reduction; the correlation with FH fell just short of significance. TSH was positively correlated with both FH and lumen size decrease, though the relationship was significant only with the former (Table 10, Figures 3E, 3F).

Rabbit Developmental Study – GD29 Dams (Argus, 1998c; York, 1998e)

The significant relationships between thyroid hormones, TSH, and measurements of standard histopathology (SH) that are present in the Developmental Neurotoxicology and Subchronic studies were not present in the rabbit teratology data set (Tables 11 and 12, Figure 4). T4 is significantly related to T3 levels and FH. TSH is not significantly correlated with FH. T4 and TSH are not significantly correlated.

Discussion

Strong correlations were seen between thyroid hormone levels, thyroid hormone and TSH levels, and thyroid hormone or TSH levels and standard ratings of thyroid histopathology. These relations were clearest in the Caldwell 14-Day study, in which strong correlations existed between the elements of thyroid hormone homeostasis feedback loop and between hormone levels and observed severity of follicular cell histopathology.

In the Subchronic Study, correlations were established between hormone levels and FH across both dosing time points and for each time point. At 14 days of dosing the expected inverse

relationship between T4 and TSH was not found. At 90 days of dosing, the inverse relationships between T3 or T4 and TSH were found, along with significant correlations of these hormone levels with severity ratings of thyroid histopathology .

Similar relationships were observed in the Developmental Neurotoxicity study with PND5 pups. T4 and TSH were significantly negatively correlated, as expected because of the negative feedback loop that exists to mediate thyroid hormone level, and T3, T4, and TSH were significantly correlated with one or the other of the histopathology endpoints.

The Rabbit Developmental study yielded significant correlations between T3 and T4 and T4 and histopathology. It did not, however, show the relationships between T4 and TSH and TSH and histopathology seen in the rat studies.

The correlations seen in the rat studies support the notion that manipulations which result in decreased levels of circulating thyroid hormone are tied to histopathological changes which are thought to directly result from elevation of TSH.

Tables

Table 1 -- Caldwell 14 -Day Study. Pearson's r correlations between thyroid hormones and TSH, n = 96.

	T3	T4	TSH
T3	1.00 p = 0.00	0.81 p = 0.0001	-0.65 p = 0.0001
T4		1.00 p = 0.00	-0.67 p = 0.0001
TSH			1.00 p = 0.00

Table 2 – Caldwell 14 -Day Study. Spearman's r_s correlations between rank order of thyroid hormone and TSH levels and FH and decrease in Lumen Area histopathology severity ratings, n = 95.

	FH	LA
T3	-0.67 p = 0.0001	-0.74 p = 0.0001
T4	-0.66 p = 0.0001	-0.70 p = 0.0001
TSH	0.71 p = 0.0001	0.79 p = 0.0001
FH	1.00 p = 0.00	0.75 p = 0.0001

Table 3 -- Subchronic study. Pearson's r correlations between thyroid hormones and TSH using data from 14 and 90 days of dosing combined, n = 223.

	T3	T4	TSH
T3	1.00 p = 0.00	0.42 p = 0.0001	-0.18 p = 0.007
T4		1.00 p = 0.00	-0.20 p = 0.0027
TSH			1.00 p = 0.00

Table 4 – Subchronic study. Spearman's r_s correlations between rank order of thyroid hormone and TSH levels and FH histopathology severity ratings using data from 14 and 90 days of dosing combined, n = 223.

	FH
T3	-0.256 p = 0.0001
T4	-0.239 p = 0.0003
TSH	0.365 p = 0.0001

Table 5 -- Subchronic study. Pearson's r correlations between thyroid hormones and TSH after 14 days of dosing, n = 104.

	T3	T4	TSH
T3	1.00 p = 0.00	0.36 p = 0.0001	-0.11 p = 0.27
T4		1.00 p = 0.00	0.20 p = 0.04
TSH			1.00 p = 0.00

Table 6 – Subchronic study. Spearman's r_s correlations between rank order of thyroid hormone and TSH levels and FH histopathology severity ratings after 14 days of dosing, n = 104.

	FH
T3	-0.24 p = 0.01
T4	-0.27 p = 0.005
TSH	0.463 p = 0.0001

Table 7 – Subchronic study. Pearson's r correlations between thyroid hormones and TSH after 90 days of dosing, n = 119.

	T3	T4	TSH
T3	1.00 p = 0.00	0.66 p = 0.0001	-0.40 p = 0.0001
T4		1.00 p = 0.00	-0.38 p = 0.0001
TSH			1.00 p = 0.00

Table 8 – Subchronic study. Spearman's r_s correlations between rank order of thyroid hormone and TSH levels and FH histopathology severity ratings after 90 days of dosing, n = 119.

	FH
T3	-0.27 p = 0.003
T4	-0.44 p = 0.0001
TSH	0.295 p = 0.001

Table 9 -- Developmental neurotoxicity study. Pearson's r correlations between thyroid hormones and TSH in PND5 pups, n = 22 - 27.

	T3	T4	TSH
T3	1.00 p = 0.00	0.87 p = 0.0001	-0.43 p = 0.03
T4		1.00 p = 0.00	-0.57 p = 0.0046
TSH			1.00 p = 0.00

Table 10 – Developmental neurotoxicity study. Spearman's r_s correlations between rank order of thyroid hormone and TSH levels and FH histopathology severity ratings in PND5 pups.

	FH	LA
T3 n = 23	-0.33 p = 0.12	-0.44 p = 0.03
T4 n = 27	-0.35 p = 0.07	-0.505 p = 0.007
TSH n = 36	0.39 p = 0.019	0.28 p = 0.10

Table 11 -- Rabbit Developmental Toxicity study. Pearson's r correlations between thyroid hormones and TSH in GD29 Dams, n = 140.

	T3	T4	TSH
T3	1.00 p = 0.00	0.52 p = 0.0001	0.20 p = 0.016
T4		1.00 p = 0.00	0.088 p = 0.302
TSH			1.00 p = 0.00

Table 12 – Rabbit Developmental Toxicity study. Spearman's r_s correlations between rank order of thyroid hormone and TSH levels and FH histopathology severity ratings in GD29 Dams, n = 140.

	FH
T3	0.034 p = 0.685
T4	-0.166 p = 0.05
TSH	0.074 p = 0.385

Figure captions:

1. Caldwell 14 day study.
   A (left). T3 and T4 hormone levels are highly positively correlated.
   B. (Right) T4 and TSH are highly negatively correlated.
   C. (Top) The rank of T4 level is highly negatively correlated with severity ratings for follicular hypertrophy (FH) (left) and decrease in follicular lumen area (LA) (right).
   D. (Bottom) The rank of TSH is highly positively correlated with severity ratings of FH (left) and LA (right).

2. Subchronic study
   A. For the period during which rats were actively dosed with ammonium perchlorate (tests at 14 and 90 days of dosing combined), T3 and T4 were highly significantly correlated (top left) and T4 and TSH were significantly negatively correlated (top right). T4 and TSH were negatively and positively correlated, respectively, with standard histopathological ratings of FH (bottom, left and right) (see legend accompanying Figures 2B and 2C).
   B. At the fourteen day timepoint, T3 and T4 are highly associated (top left). T4 and TSH are, unexpectedly, positively correlated (top right). T4 and TSH are negatively and positively correlated, respectively, with FH (bottom left and right).
   C. At the 90 day time point, there are strong correlations between T3 and T4 (top left), T4 and TSH (top right), and T4 or TSH and FH severity ratings (bottom left and right).

3. Developmental neurotoxicity study, postnatal day five (PND5) pups.
   A. T3 and T4 are strongly positively correlated.
   B. T4 and TSH are strongly negatively correlated.
   C and D. T4 is negatively associated with both FH and reduction in LA, but the correlation reaches significance only for LA.
   E and F. TSH is positively correlated with FH and LA, but the correlation reaches a significance level only with FH.

4. Rabbit Developmental Toxicity study, gestational day 29 dams. T3 and T4 are strongly positively correlated (top left). There is no significant correlation between T4 and TSH (top right). TSH is not correlated with FH (bottom right), while T4 is borderline significant with FH (bottom left).

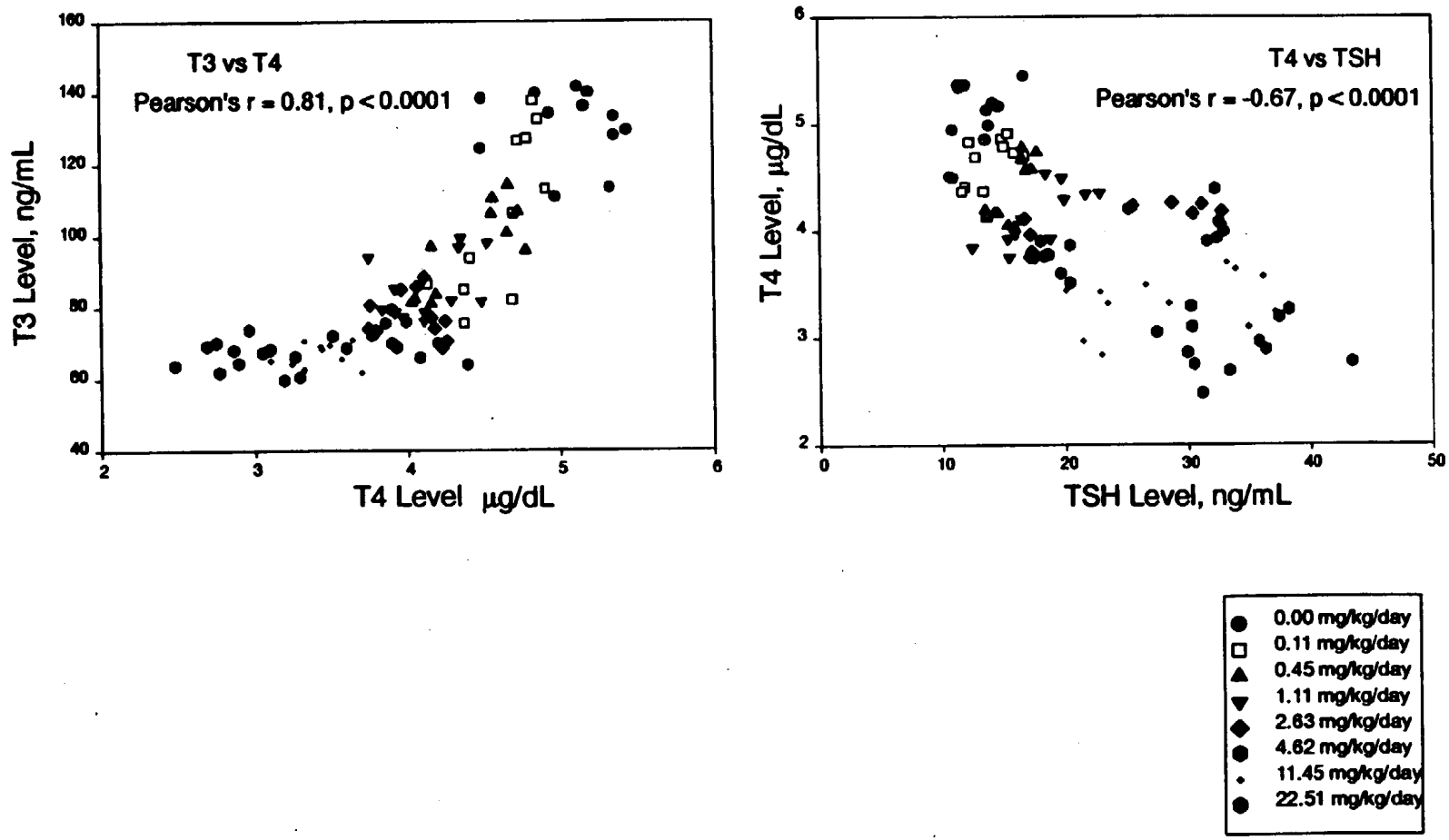


Figure 1A (left) and 1B (right)

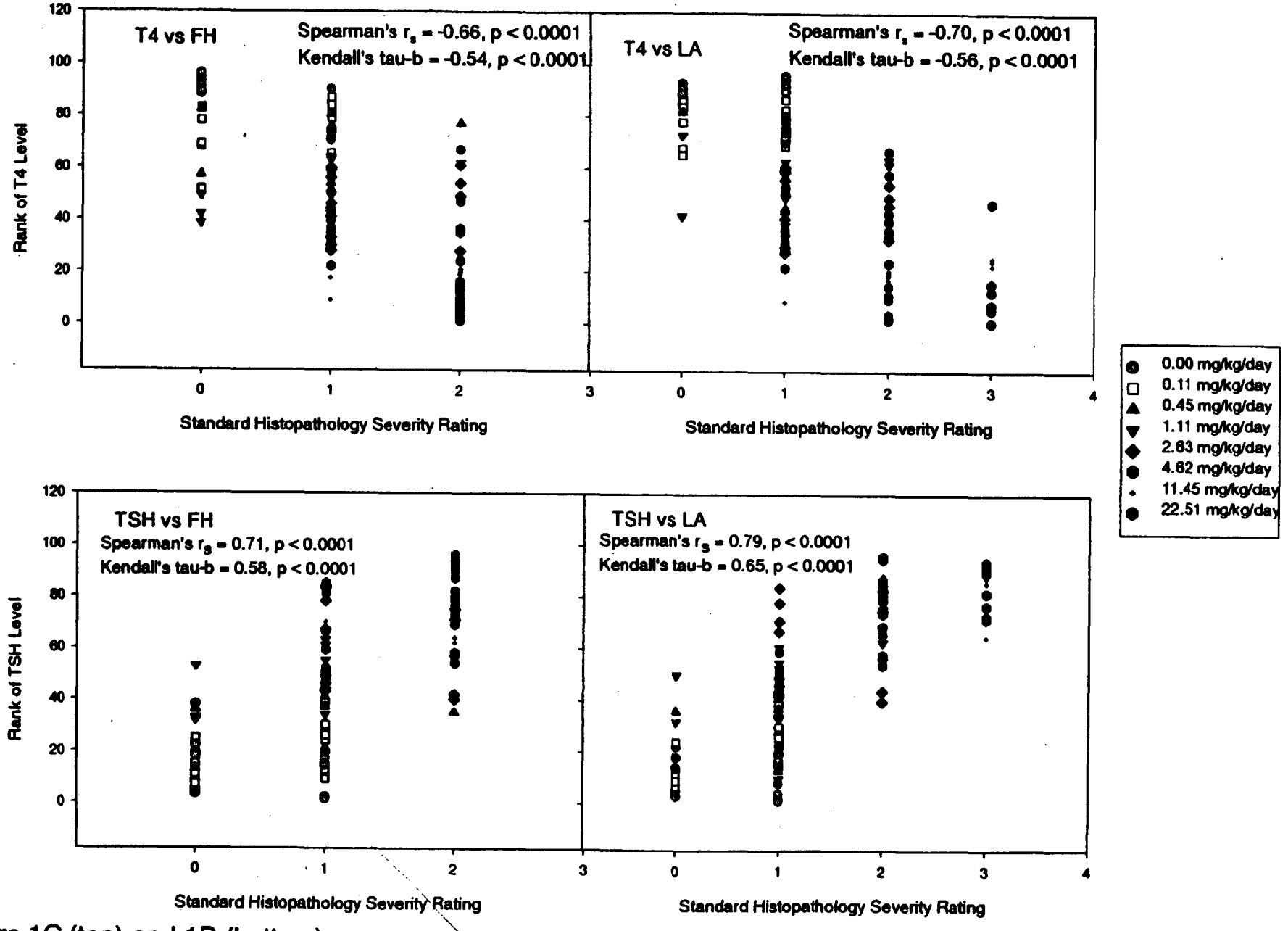
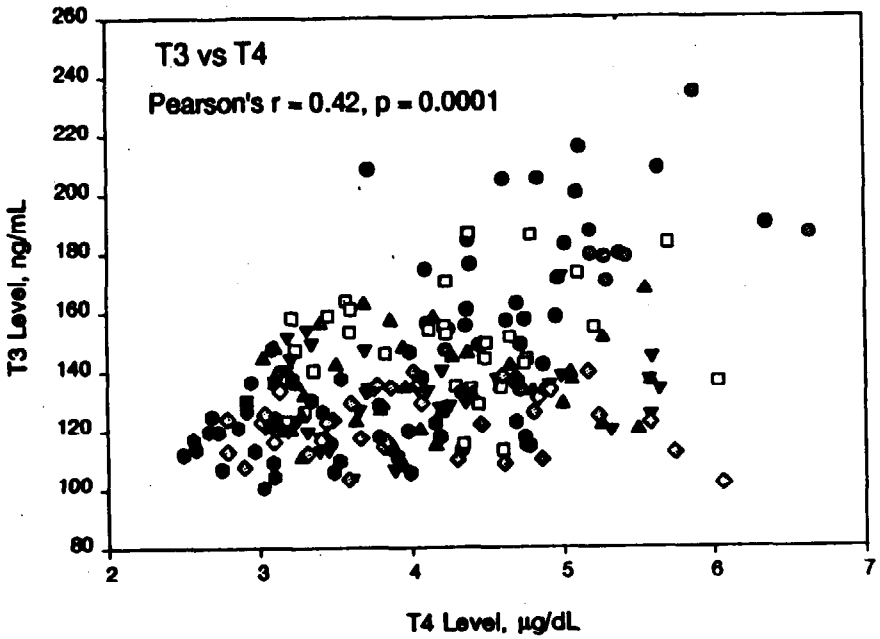
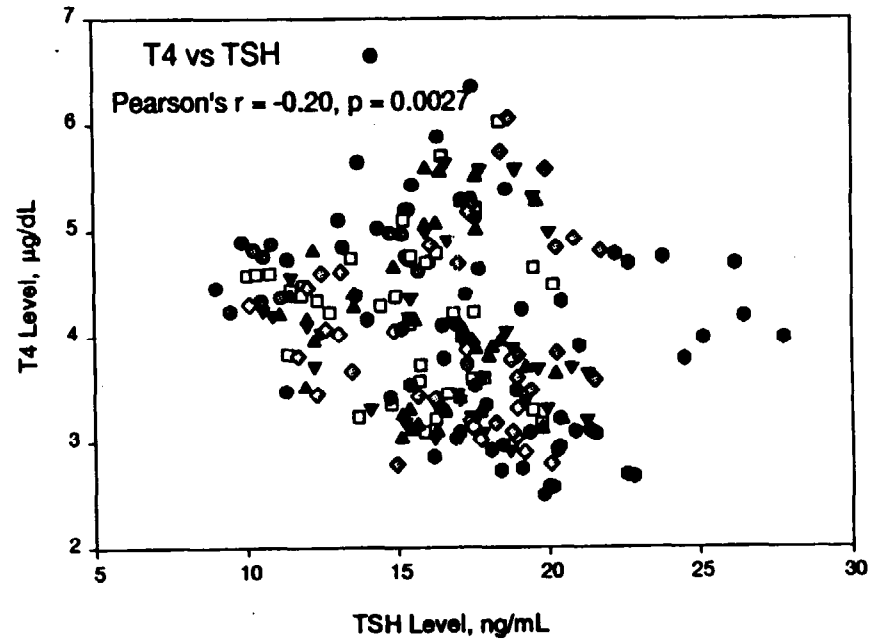


Figure 1C (top) and 1D (bottom)

Subchronic Study, 14 and 90 Day Time Points Combined





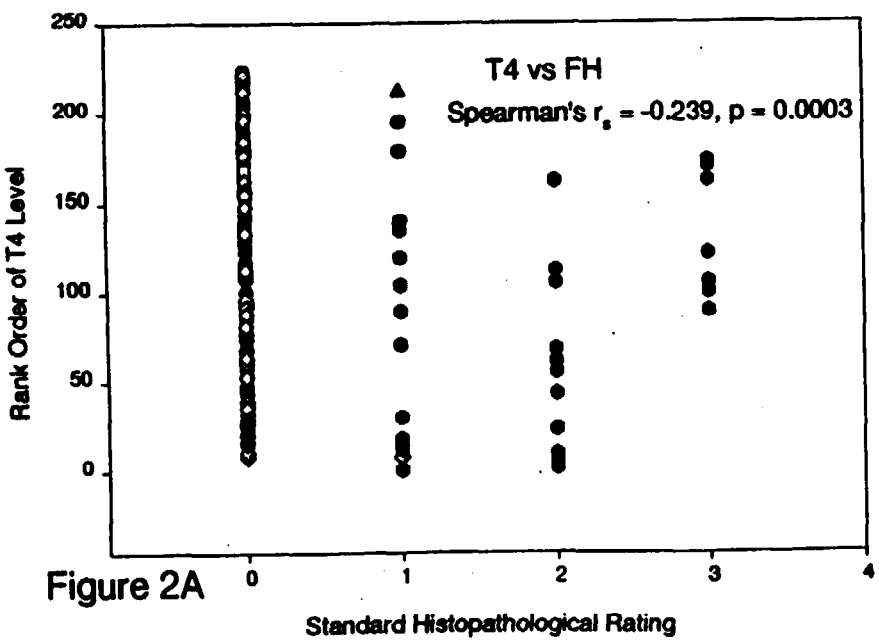
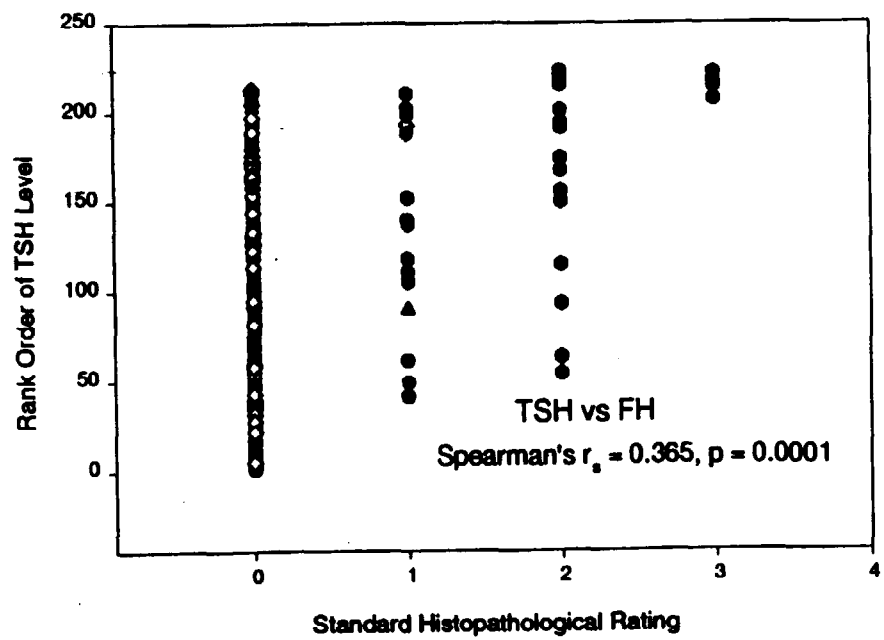
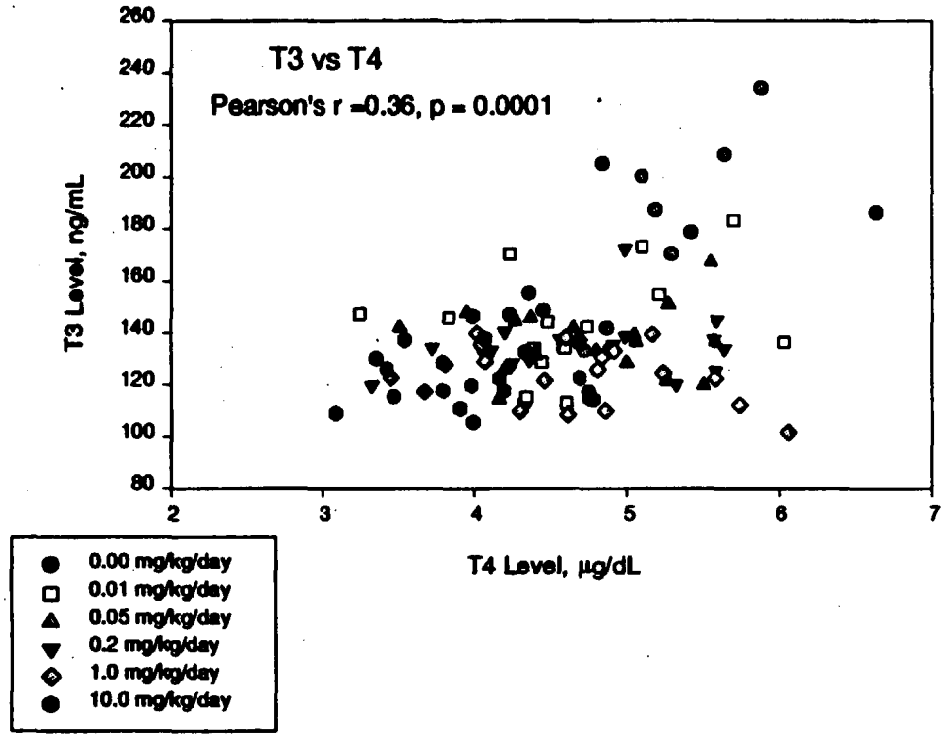
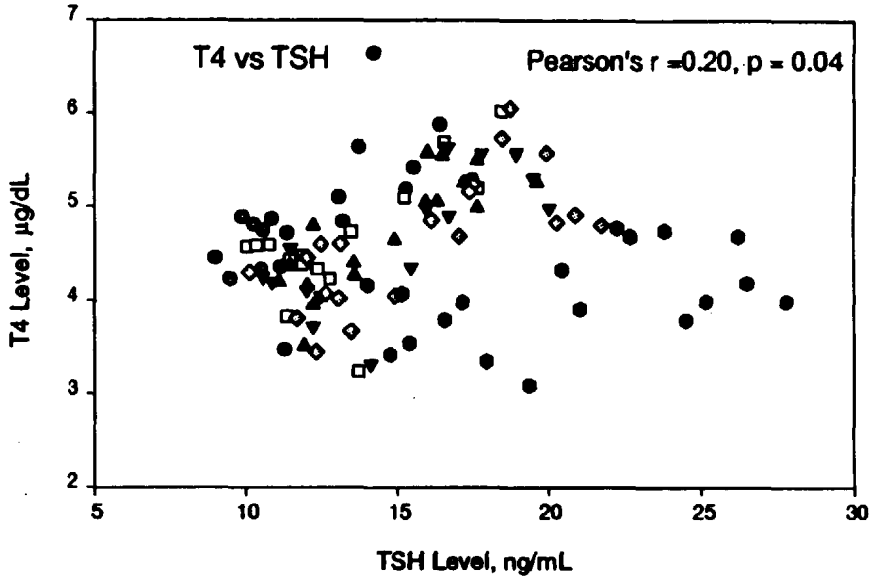


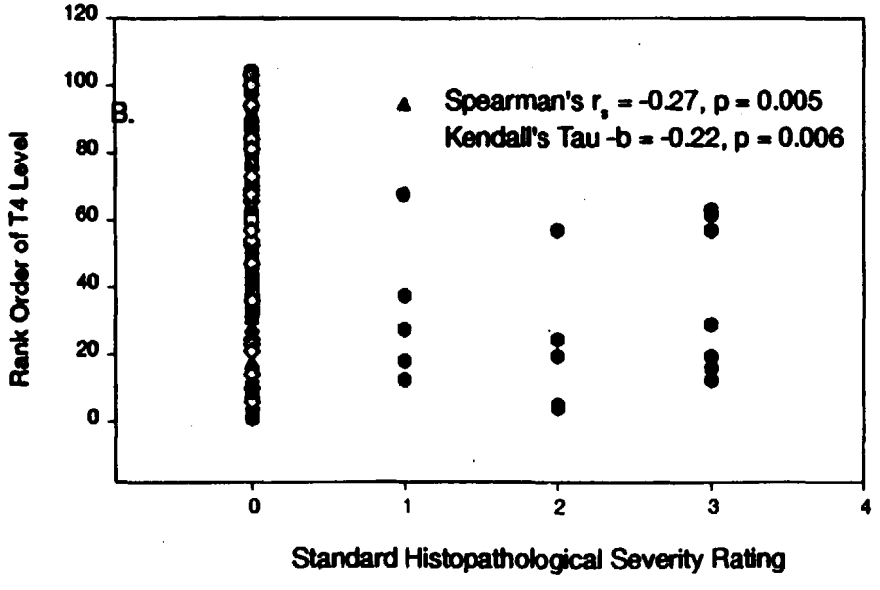
Figure 2A



Subchronic Study, 14 Day Time Point







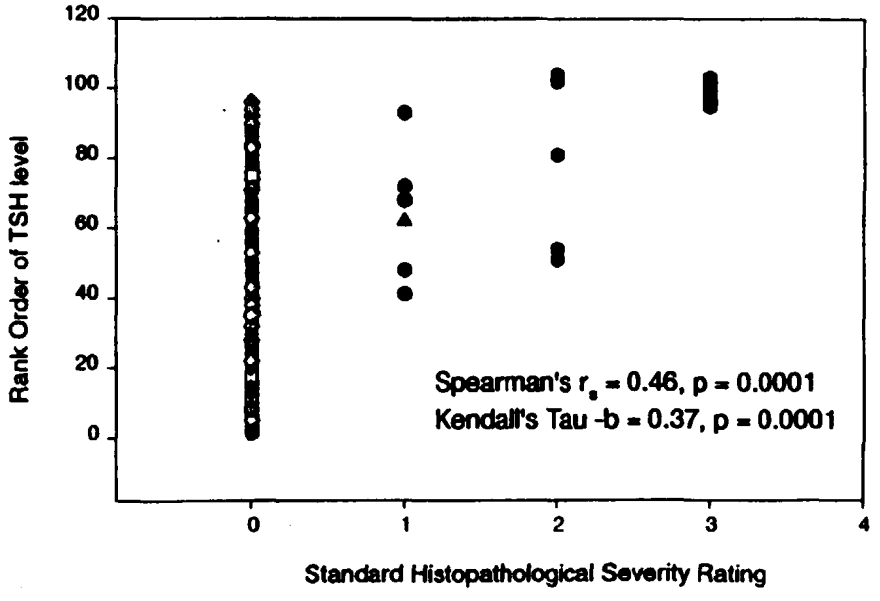
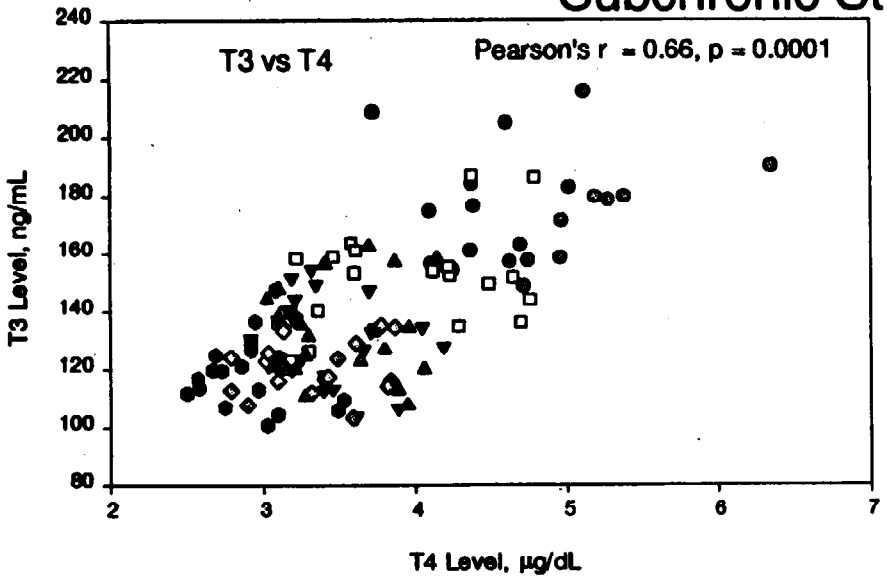
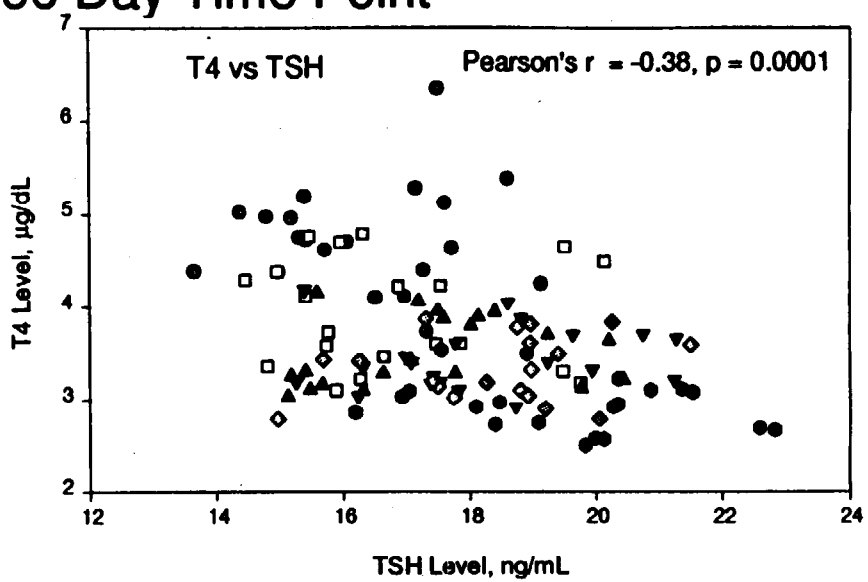
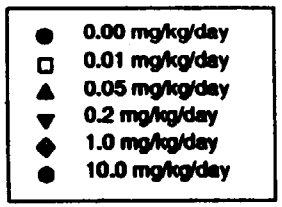


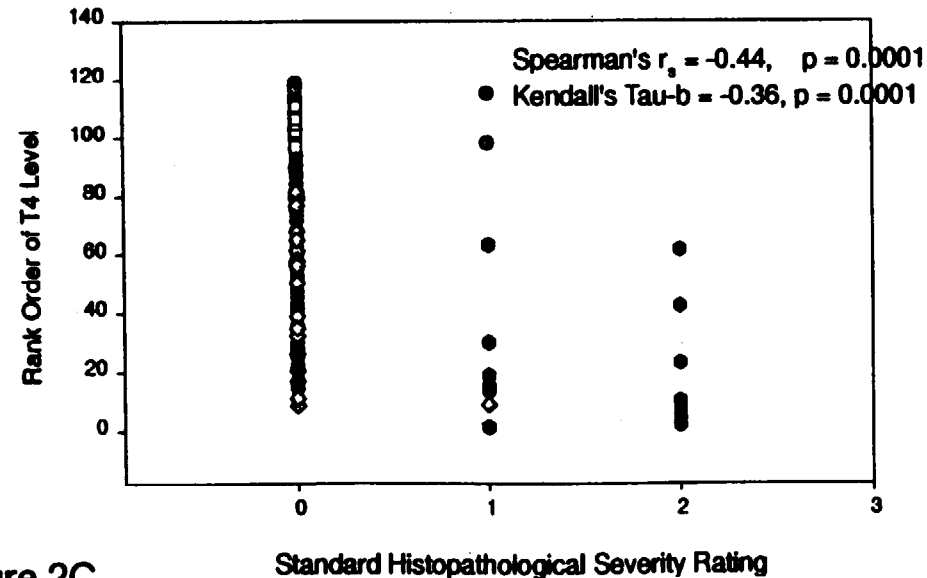
Figure 2B

Subchronic Study, 90 Day Time Point









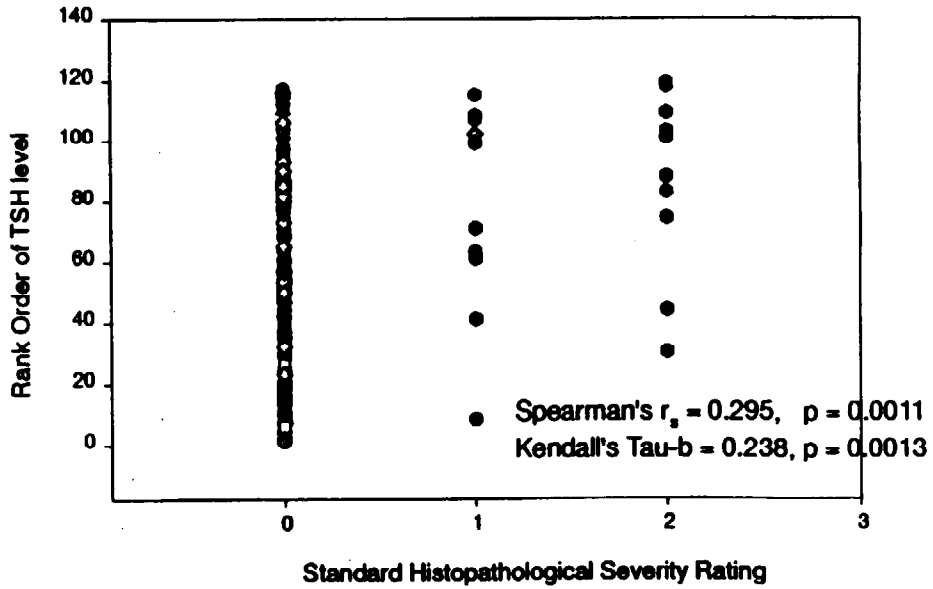


Figure 2C

Developmental Neurotoxicity, PND5

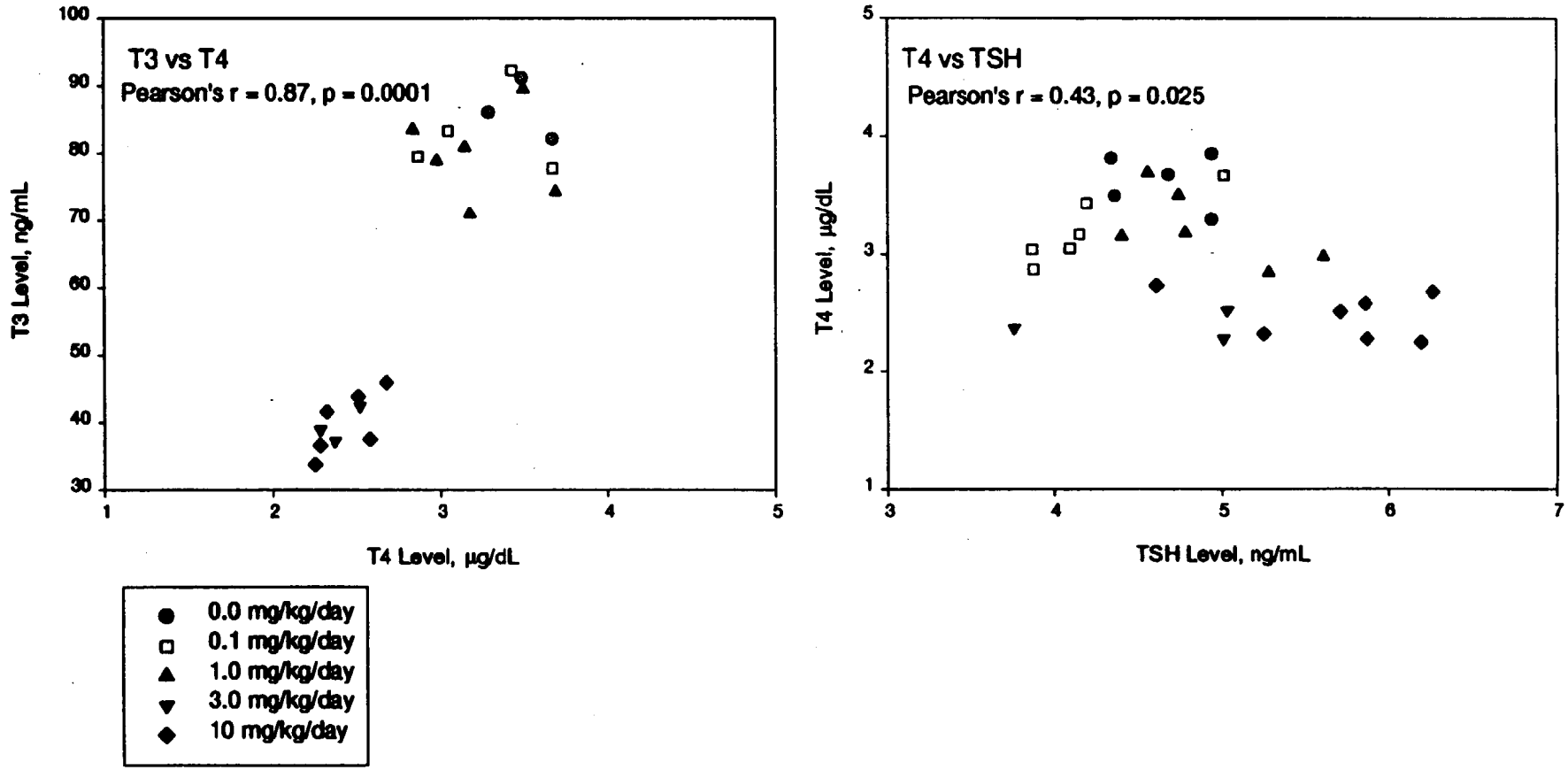
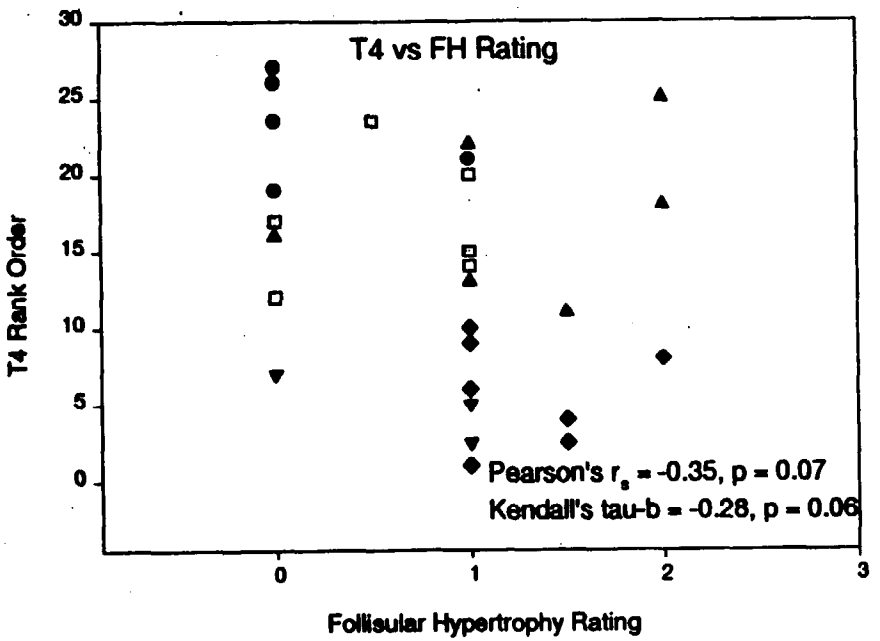
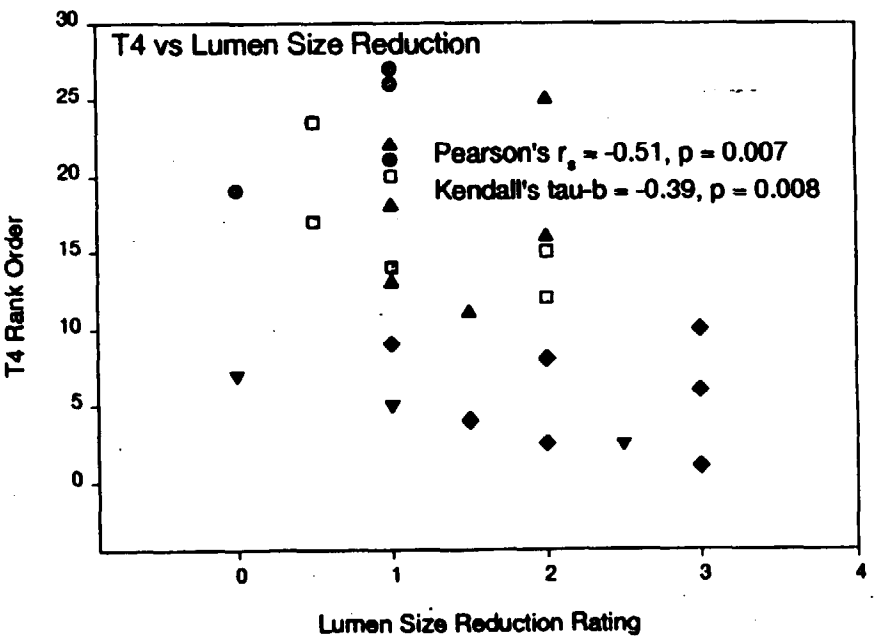


Figure 3A (left) and 3B (right)

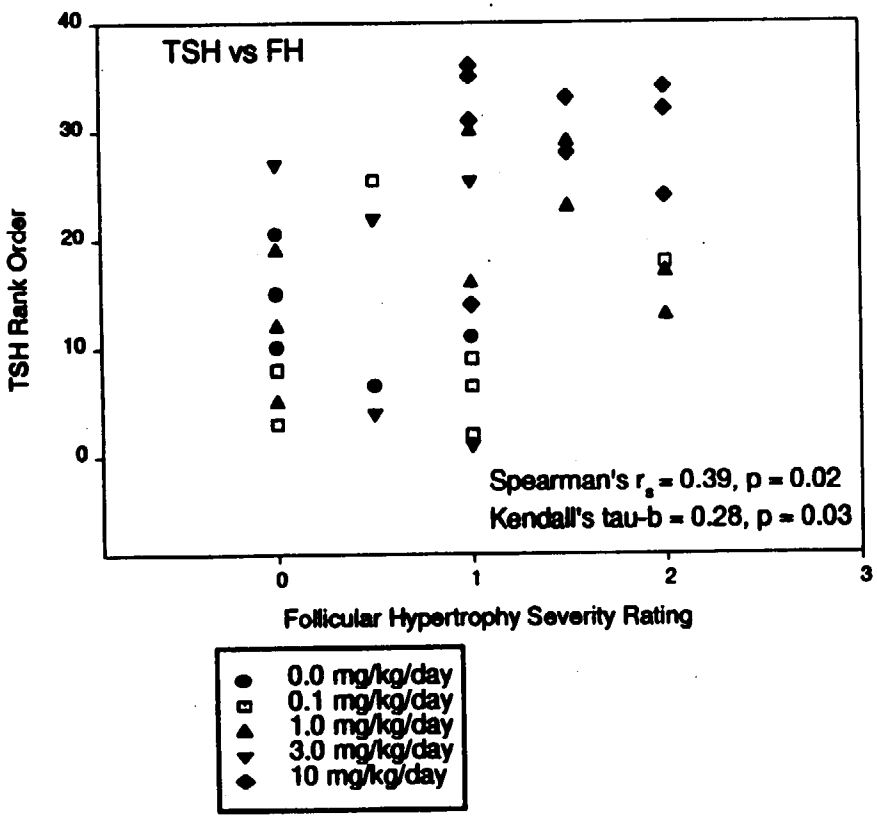
Developmental Neurotoxicity, PND5





Figures 3C (left) and 3D (right)

Developmental Neurotoxicity, PND5



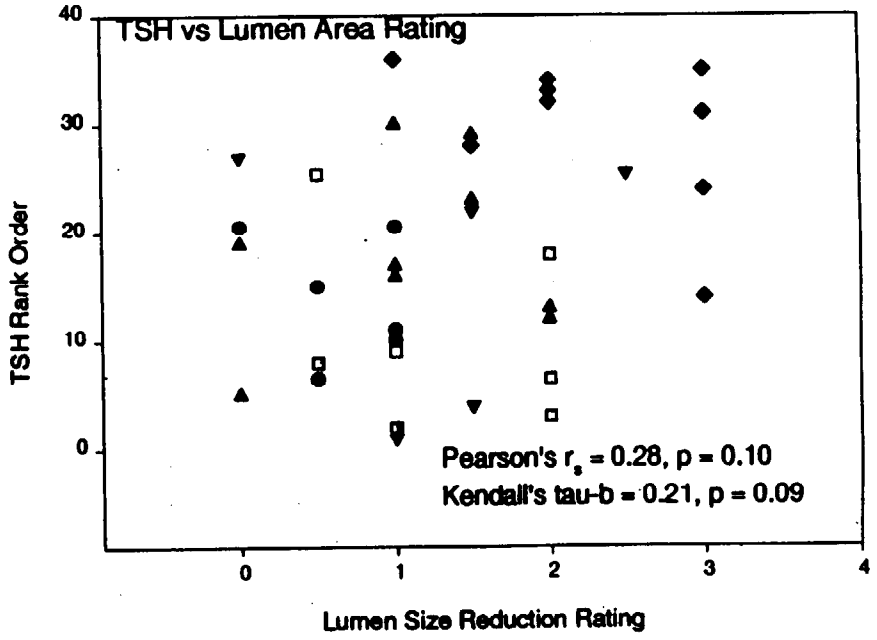
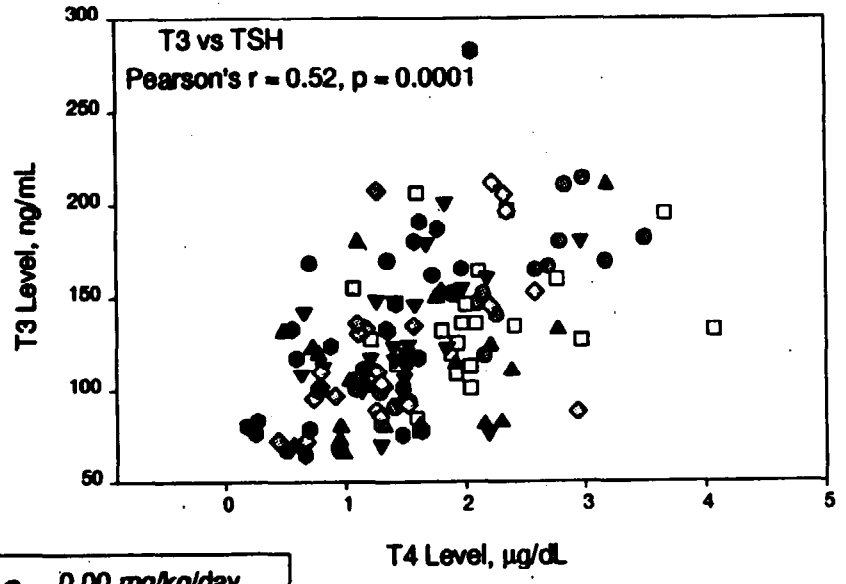
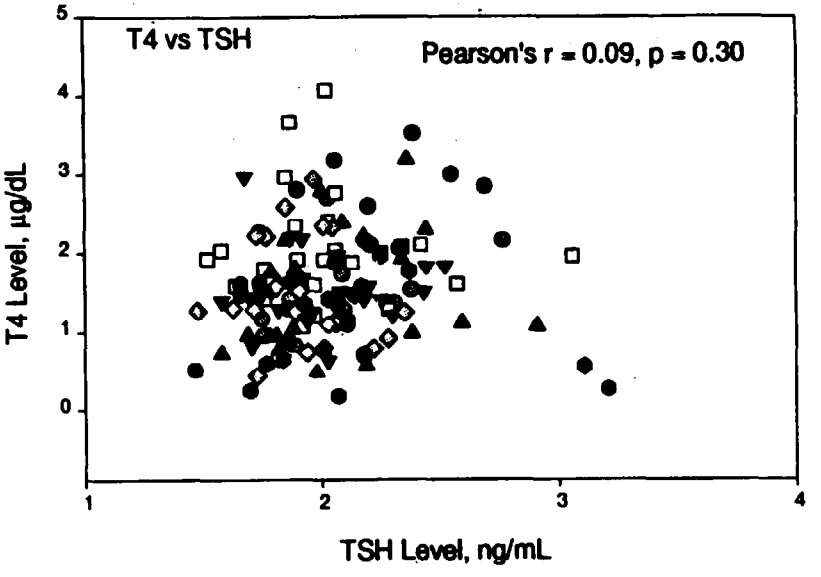
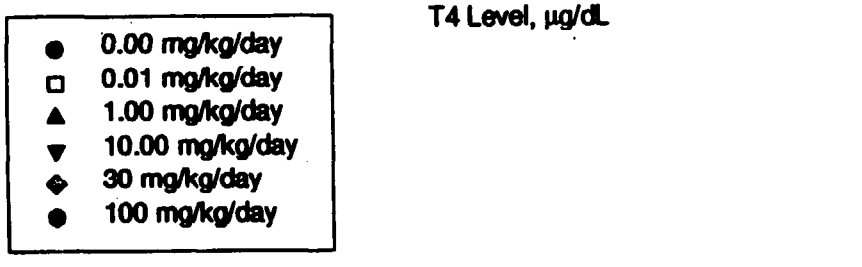


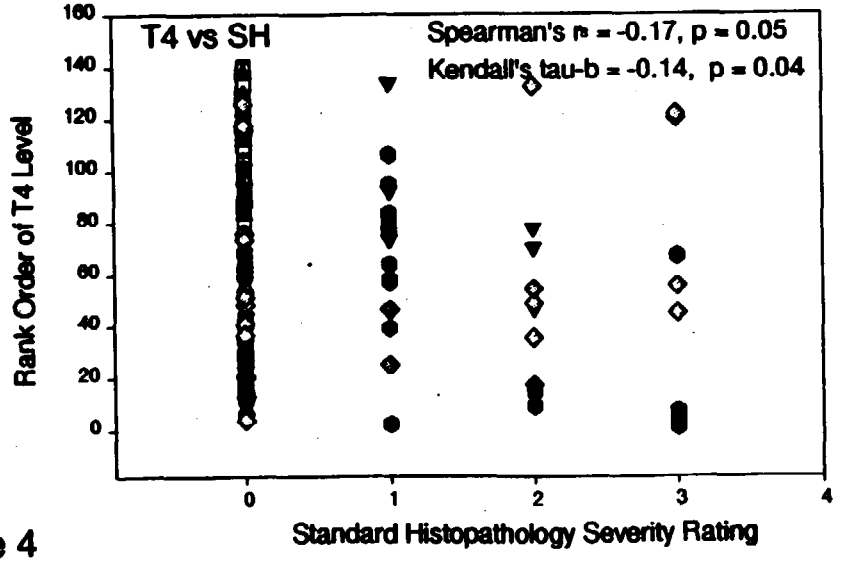
Figure 3E (left) and 3F (right)

Rabbit Developmental Study, Dams, GD 29









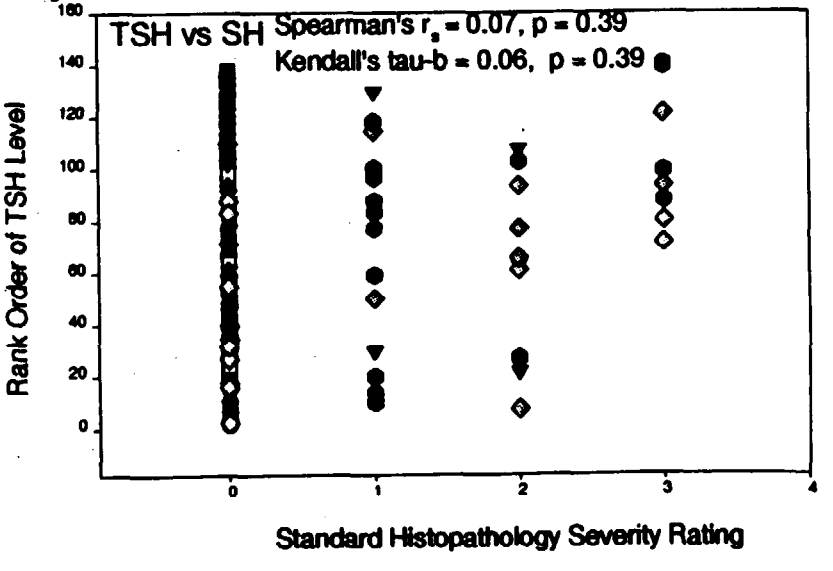


Figure 4